10/089841

JC18 Rec'd PCT/PTO 04 APR 2002

Attny Docket No. B1180/20005
PTO Customer No. 03000

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

International Application No.	:	PCT/EP00/09808
International Filing Date	:	October 6, 2000
Priority Dates Claimed	:	October 6, 1999 (DE 199 48 087.7)
Title of Invention	:	STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME
Applicant(s) for DO/EO/US	:	Susanne BRAKMANN Helmut PEUKER Wolfgang SIMM Ulrich KETTLING Andre KOLTERMANN Jens STEPHAN Thorsten WINKLER Klaus DÖRRE Manfred EIGEN

Box PCT (EO/US)
Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. This is an express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

3. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

4. The International Application as filed (35 U.S.C 371(c)(2)) has been transmitted by the International Bureau. A copy of the cover sheet of the international application as published on April 12, 2001, under International Publication No. WO 01/24933 is attached hereto.

5. A copy of the English translation of the application as originally filed is enclosed.

6. Nine (9) sheets of formal drawings are enclosed.

7. A Preliminary Amendment and an Abstract of the Disclosure are enclosed.

8. An Information Disclosure Statement, PTO Form 1449 and copies of each of the references disclosed therein are enclosed.

9. Calculation sheet, in duplicate, is attached.

This application and items attached are being transmitted within the 30 month claimed priority date.

Respectfully submitted,

CAESAR, RIVISE, BERNSTEIN,
COHEN & POKOTILOW, LTD.

April 4, 2002

By ______________________
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CALCULATION SHEET
BASIC NATIONAL FEE (37 CFR 1.492(1)(1)-(5):

Claims Fee

	Claims Filed	Extra Claims	Rate	Calculation
Total Claims	17	0	x 18	$
Independent Claims	1	0	x 84	
Multiple Dependent Claims			x280	

Basic Fee U.S. PTO was not International Preliminary Examination Authority. Search report on the international application was prepared by the European Patent Office — $ 890.00

Total

Reduction by ½ for filing by small entity

TOTAL NATIONAL FEE **$ 890 .00**

Please charge counsel's account no. 03-0075 in the amount of $890, or any additional amount which may be required, to cover the above fees. A duplicate copy of the calculation sheets is enclosed.

AUTHORIZATION TO CHARGE ADDITIONAL FEES

The Commissioner is hereby authorized to charge the following additional fees which may be required by this paper and during the entire pendency of this application to counsel's deposit account:

1. 37 CFR 1.492(a)(1), (2), (3) and (4) (filing fees)
2. 37 CFR 1.492(b), (c) and (d) (presentation of extra claims)
3. 37 CFR 1.17 (application processing fees)
4. 37 CFR 1.492(e) and (f) (surcharge fee for filing declaration and/or filing an English translation of an International Application later than 30 months after the priority date)

03-0075
Deposit Account

April 4, 2002
Date

David M. Tener - Reg. No. 37,054

Attorney Docket No. B1180/20005

10/089841

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Attny Docket No. B1180/20005
PTO Customer No. 03000

Applicants	:	Susanne BRAKMANN et al
Title	:	STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME

The following documents are submitted for the above-identified invention for entry into the U.S. National stage under 35 U.S.C. §371, based on the International Application No. PCT/EP00/09808, which includes the following:

1. Application Data Sheet;
2. Two (2) sheets of Transmittal Letter;
3. Calculation sheet, in duplicate;
4. English translation of Application as filed;
5. Nine (9) sheets of formal drawings;
6. Preliminary Amendment and attached Abstract
7. Cover sheet of International Publication;
8. Information Disclosure Statement, PTO Form 1449 and copies of each of the references disclosed therein; and
9. Return Receipt Post Card

"Express Mail" Mailing Label No. EL 932 778 652 US
Date of Deposit April 4, 2002

I hereby certify that the above-identified documents are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to BOX PCT, Commissioner for Patents Washington, D.C. 20231, Attention RO/EO/US

Judith Perez

Judith Perez

JC13 Rec'd PCT/PTO 04 APR 2002

APPLICATION DATA SHEET

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Attorney Docket Number: **B1180/20005**

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Title of Invention: **STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME**

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9-0

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Susanne BRAKMANN et al.

Serial No: Group Art Unit:

Filed: April 4, 2002 Examiner:

Att. Docket No.: B1180/20005 Confirmation No.:

For: STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Prior to initial examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Page 1, above line 3 and below the title, please insert the following centered heading:

-- BACKGROUND OF THE INVENTION --.

Page 4, please delete the last paragraph.

Page 5, please insert the following centered heading above line 1:

-- SUMMARY OF THE INVENTION --.

Page 12, between paragraphs 2 and 3, please insert the following centered heading:

-- BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS --.

Page 13, between paragraphs 7 and 8, please insert the following centered heading:

-- DETAILED DESCRIPTION OF THE INVENTION --.

IN THE CLAIMS:

Please cancel claims 1-16 without prejudice or disclaimer.

Please add claims 17-33 as follow:

-- 17. A reaction substrate comprising:

a base having a substantially planar smooth surface and comprising at least one base material selected from the group consisting of glass, plastic, metallic and semiconductor, and

a flexible compartment layer comprising a viscoelastic polymer composition perforated by an arrangement of holes, wherein the flexible compartment layer is adapted to removably adhere to the surface of the base such that the flexible compartment layer can be separated from the base substantially free of damage and without loss of form, adhesion and flexibility, and wherein the holes combine with the base to provide sample reservoirs when the flexible compartment layer is adhered to the base, such that the surface of the base acts as a floor for each of the sample reservoirs.

18. The reaction substrate of claim 17, wherein the base comprises a transparent material.

19. The reaction substrate of claim 18, wherein the base is a substantially planar smooth glass plate.

20. The reaction substrate of claim 19, wherein the glass plate has a thickness of a cover glass used in microscopy.

21. The reaction substrate of claim 20, wherein the thickness is about 150 μm.

22. The reaction substrate of claim 17, wherein the viscoelastic polymer composition comprises natural or synthetic rubbers free of adhesive and solvent.

23. The reaction substrate of claim 22, wherein the viscoelastic polymer composition comprises silicon rubber.

24. The reaction substrate of claim 22, wherein the viscoelastic polymer composition adheres to the base without adhesive.

25. The reaction substrate of claim 17, further comprising a cover mountable on a side of the flexible compartment layer opposite to the base.

26. The reaction substrate of claim 25, wherein the cover has penetration openings for supplying samples to the sample reservoirs or for removing samples from the sample reservoirs.

27. The reaction substrate of claim 17, wherein the flexible compartment layer further comprises channels and/or storage pots.

28. The reaction substrate of claim 17, wherein the flexible compartment layer further comprises fluid lines, electrodes and/or sensors.

29. The reaction substrate of claim 17, in a form of a microtiter or nanotiter plate.

30. The reaction substrate of claim 17, wherein variations of positions of the sample reservoirs in a direction vertical to a plane of the reaction substrate over the entire surface of the base are less than 250 μm.

31. The reaction substrate of claim 30, wherein the variations are less than 150 μm.

32. The reaction substrate of claim 30, wherein the variations are less than 100 μm.

33. The reaction substrate of claim 17, adapted for:

identifying and characterizing synthetic or biological objects;

identifying and characterizing chemical compounds;

identifying and/or validating targets;

searching for biologically active substances and/or pharmaceutical substances;

identifying conductive structures;

genome analysis;

proteome analysis;

cleaning and concentrating substrates; or

evolutive optimizing of biologically relevant macromolecules. --

Attorney Docket No. B1180/20005

REMARKS

By this Amendment, the specification is amended, claims 1-16 are cancelled and claims 17-33 are added. Claims 17-33 are pending.

This Amendment is submitted to better conform the application to United States practice by, *inter alia*, eliminating multiple dependencies from the claims and adding section headings to the specification.

It is respectfully submitted that the application is in good form for initial examination on the merits. Accordingly, prompt and favorable examination on the merits is respectfully requested.

Should the Examiner believe that anything further is desirable in order to place the application in even better condition for initial examination and allowance, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,

CAESAR, RIVISE, BERNSTEIN,
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By____________________
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April 4, 2002

Please charge or credit our Account No. 03-0075 as necessary to effect entry and/or ensure consideration of this submission.

10/089841
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9/prts

STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME

The present invention relates to a reaction substrate for accomodating and/or manipulating a plurality of separate samples, in particular a structured reaction substrate for microscopically small sample quantities, a process and a tool for its production and also uses of the reaction substrate.

In biochemistry, medicine and genetics there is a wide need for processes for manipulating, observing and/or analysing a plurality of samples. Test procedures with high sample throughput (so-called high throughput screening, HTS) were developed, where thousands of samples are isolated for example, cultivated or subjected to certain treatment in parallel. These methods are carried out in specially adapted reaction substrates or containers having many sample compartments, which must satisfy a large number of requirements. For example, the reaction substrates must ensure rapid and parallel supply of samples, observation of the sample during reaction and further availability of the sample after reaction and must be inert relative to each reaction. With the advance in the level of knowledge in biochemistry and improvements in methods originating from biotechnology and combinatory chemistry there is the necessity to process as large a number as possible of the smallest possible sample volumes in parallel, that is, be able to handle, control and measure them. In recent times on the basis of modern (fluorescence-) screening techniques per day 10^3 to 10^5 samples can be characterised in required volumes of 10^{-6} to 10^{-10} l. To increase the sample throughput, reduce the substance usage and also for space reasons a particular aim here is to miniaturise the sample compartments. The demands on sample carriers with compartmenting for individual samples are also increasing directly and sharply. This applies in particular with

respect to the number of available compartments, the miniaturisation potential, easy handling and costs or reusability.

Sample carrier or reaction substrates with microscopically small structures for use in fluorescence, luminescence or scintillation measuring, e.g. for solving chemical or molecular-biological issues, are known per se. DE-OS 197 12 484, EP 131 934, US 54 17 923 and US 54 87 872 describe reaction substrates in the form of structured microplates each of which forms a plurality of sample compartments arranged flat and open on one side. A microplate with a filter membrane is described in EP 408 940. Due to its complicated structure this microplate is disadvantageous both for manufacturing and for cleaning. The number of available compartments is limited.

Another microstructured reaction substrate is described in WO 95/01559. Recesses whose bases are at least partially porous towards the underside are formed on the surface of the sample carrier made of a semiconductor material or a synthetic material by etching. These reaction substrates enable examination from both sides. But they embody disadvantages with respect to reproducibility of manufacture of individual recesses and handling capacity of the reaction substrate. If there is a cover provided for the recesses, it must be attached separately and mechanically by clamping, gluing or bonding.

DE-OS 197 52 085 discloses a simple-to-manufacture reaction substrate for microscopic examinations of a plurality of samples, which has a substrate with sample compartments formed by casting technology and/or hot stamping. One disadvantage of this sample carrier is that microscopic examinations can be made from one side only of the substrate, on which the sample compartments are open. In

addition, this reaction substrate cannot generally be used for HTS processes.

WO 99/19717 discloses the construction of a microsystem, wherein at least one flexible, microstructured film is arranged as a laminate between solid carriers. The film has application-specific microstructures, into which electrodes are integrated if required, and which form compartments for liquid samples in conjunction with the carriers. This stacking technique is once again disadvantageous, since separate measures have to be taken for connecting the carriers to the film, which influence handling of the samples or the samples themselves.

A similar structure is described in EP 324 153. In particular a photopolymer provided with certain microstructures is laminated to a solid carrier as a layer. The disadvantage of this technique is that the polymer layer cannot be removed from the carrier without damage being done. But there is interest in reaction substrates or sample carriers which can be detached from a substrate compound for reuse or for additional procedural steps for processing samples, for example without damage thereto.

A method for producing microstructures on a metal surface is described in WO 97/29223. The metal surface is worked by a photolithographically structured polymer layer. But use of this technique does not solve the problem of covering microstructures. Other structuring techniques for materials made of metal or semiconductors are described in EP 869 556, WO 97/13633 and WO 98/09745.

A general disadvantage of the conventional reaction substrates for use in microscopy is their relatively thick, uneven and/or sagging bases. The bases of conventional reaction substrates can comprise various materials such as glass. Typical glass thicknesses are around 500 μm. But

there can also be distinctive, irreproducible variations of the base (e.g. over 400 µm). The focal length of immersion lenses is typically limited to 250 to 300 µm, however. When the cover glass thickness of around 150 µm is substracted there is still a permissible variance of the base of around 100 to 150 µm, to be able to make reproducible, continuous measurements on the reaction substrate without constant readjustments to the position of the lens in a direction vertical to the plane of the reaction substrate (hereinbelow designated as the z direction).

In most of the abovementioned requirements, but also with respect to reliability and reproducibility, the previously available reaction substrates or containers or sample carriers cannot endure with the development of screening technology.

It is the object of the present invention to provide an improved reaction substrate with which the disadvantages of conventional reaction substrates can be avoided and which in particular has a simple structure, is inert under the interesting reaction conditions and is also simple to manufacture with any structures and easy to handle. The new reaction substrate should in particular also be reusable several times or recyclable. The object of the invention is in particular to provide an improved reaction substrate, which facilitates handling and examining samples, e.g. with a microscope. The object of the invention is also to provide a process for producing the reaction substrate and a tool for carrying said process into effect.

These objects are solved in particular by a reaction substrate having the characteristics of Claim 1. Advantageous embodiments and applications of the invention will emerge from the dependent claims.

According to the invention, a structured reaction substrate is provided which is formed by composition of a sample carrier (compartment layer) described below with a solid base part, to which the sample carrier adheres by itself. The base preferably comprises glass, plastic, metal or a semiconductor material. It forms a flat, smooth surface to which the sample carrier is adhered.

A particular advantage of the reaction substrate according to the present invention is that the compartment layer can be separated from the base part substantially free of damage. This means that the compartment layer can be removed from the base plate (e.g. a cover glass) such that it can be subsequently reused without substantial loss of form, adhesion and/or flexibility. For reuse purposes the compartment layer is attached to a new or cleaned base plate by light, e.g. manual, pressure to form a new reaction substrate, whose tightness corresponds fully to the tightness of the reaction substrate previously formed with the compartment layer. Removing the compartment layer substantially damage-free means that the functionality of the compartment layer remains invariably intact by its removal for later use.

The compartment layer can be lifted off the base part preferably by lifting the compartment layer from the base part at one corner, while the latter is held at its four corners. The compartment layer is bent up at the raised corner and peeled back over the base part, whereby the compartment layer is separated from the base plate substantially free from residue. A particular advantage of the invention is that such lifting and subsequent reuse can be performed as often as possible. Trials confirmed that substrates could be reused 50 times without loss of function.

According to a preferred embodiment of the invention the reaction substrate is designed for microscopic examinations. The base part comprises a transparent material (e.g. glass) having a thickness selected in dependence on application. The sample carrier is preferably applied to a cover glass used in microscopy known per se.

The thickness of the cover glass is preferably a few hundred micrometers (µm), particularly preferably around 150 µm. The microscope being used is preferably a confocal microscope. The confocal microscope is preferably combined with detection technologies which are based on the detection of fluorescence. The reaction substrates according to the present invention are particularly well suited to fluorescence correlation spectroscopy, fluorescence coincidence analysis, fluorescence distribution analysis, fluorescence lifetime measurement, fluorescence energy transfer analysis or fluorescence polarisation measurement using confocal microscopes. The reaction substrates according to the present invention are thus well suited to single molecule detection.

According to an important aspect of the invention a sample carrier is provided in the form of a flexible compartment layer with recesses for forming a predetermined compartment structure, wherein the compartment layer consists of a viscoelastic polymer composition which can independently adhere to glass, plastic, metal or semiconductor substrates. The compartment layer is a dimensionally stable mat which can be produced by means of a simple pressing procedure, whose material causes an adhesive connection (e.g. via electrostatic or von der Waals forces) with one of the abovementioned substrates with just a light manual pressure of a few grams per cm^2. The compartment layer preferably comprises substantially solvent-free natural or synthetic rubbers or compositions thereof. The compartment layer is preferably free of additives such as resins,

plasticisers and/or antioxidants. In accordance with a preferred embodiment the compartment layer of the reaction substrate according to the present invention comprises silicon rubber.

The recesses for forming the compartment structures are holes passing through the compartment layer or depressions incorporated into the compartment layer on one side. Closed compartment structures are formed in the form of sample reservoirs or storage pots and/or open compartment structures in the form of channels running in the layer plane of the sample carrier. The sample reservoirs, storage pots and channels are designated hereinbelow also as sample compartments.

The compartment structures form a plurality of matrix-like recesses arranged in straight rows and columns, whereby the grid of the matrix arrangement corresponds to the arrangement of sample reservoirs (so-called wells) of microplates and nanotiter plates.

According to another preferred embodiment of the reaction substrate according to the present invention the compartment layer is equipped with manipulation and examination instruments. These include in particular fluid lines for supplying the sample chambers formed by the compartment structures or for removing substances therefrom, sensor devices for capturing preset sample properties in the sample chambers, piezopumps for supplying liquid streams and/or electrode devices which are designed to subject the samples in the sample chambers to electrical fields. A fluid line is formed for example by a capillary running in the layer plane of the compartment layer, which extends from the edge of the sample carrier in the latter to a particular sample chamber. Sensors may comprise temperature, pH or conductivity sensors. The electrode

devices are formed preferably by electrode strips which extend in the walls of the sample chambers.

The compartment structures in a sample carrier according to the present invention form microstructures with characteristic dimensions of the order of 500 nm to 1.5 mm according to preferred embodiments.

The stacking arrangement of base part and sample carrier can be modified according to the present invention to the extent that a cover, which again is fixed by automatic adhesion relative to the sample carrier, is applied to the sample carrier on the side opposite to the base part. The cover can be formed from a rigid material such as the base part or by a flexible foil. The cover can also have predetermined openings for accessing the compartment structures. The stacking arrangement in sandwich form lends the reaction substrate additional stability. The cover serves to prevent introduced liquids from evaporating.

Depending on use it can also be arranged for several of the abovedescribed sample carriers to be disposed on a common base part to form a reaction substrate according to the present invention. Several compartment layers can therefore be attached to one another by adhesion as a stack to build up a three-dimensional fluidic system.

According to another aspect of the invention a process for producing the abovedescribed sample carrier is provided. For this an impression tool is filled with the respectively desired synthetic material of the compartment layer in a dissolved state and then the solvent is removed from the filling by tempering or drying, preferably at room temperature. The impression tool in particular comprises a structured base plate and a counter plate which are held together in a liquid-tight manner. The base plate bears projecting structures corresponding to the desired

compartment structures in the reaction substrate. These protruding structures project from the base plate depending on required use as far as or into the counter plate (formation of throughholes) or up to a level at a distance from the counter plate (formation of recesses). The counter plate is preferably provided with a coating facing the base plate, made of PTFE, for example, for repeated production of structures in the form of throughholes. The individual components of the impression device are composed of detachable plug-in or screw connections. After the solvent is removed these connections are loosened and the dried solid, dimensionally stable compartment layer is removed from the impression tool as a sample carrier. A subject of the invention is also the construction of the impression tool as such.

The sample carriers or the reaction substrate according to the present invention are designed for handling and/or examining any liquid samples with characteristic sample volumes, e.g. of the order of 1 nl to 10 µl. The fluid samples may in particular comprise solutions of predetermined reaction partners and/or suspensions containing synthetic or biological objects in a suspension fluid. Included in the objects handled in a sample carrier in particular are solid particles (so-called beads) as synthetic objects and biological cells or cell constituents, microorganisms, viruses and biologically relevant macromolecules as biological objects.

The invention has the following advantages. The reaction substrates according to the present invention can be mass-produced by simple means with a tool which operates without pressure. The sample chambers of macro to nano sizes can be easily designed in any format by way of the design of the mask or impression shape of the tool. Processing techniques for glass or semiconductors known per se, such as e.g. the LIGA process or conventional etching, are available for

producing masks for microscopically small compartment structures. The compartment structures can be manufactured highly precisely over the entire thickness of the compartment layer. In the layer plane the structures can have characteristic dimensions in the sub-micrometer range and perpendicularly thereto in the mm range.

The compartment structures can be designed in any format, such as round, square, rectangular or in a complicated geometric form. There are a plurality of advantages with regard to manufacturing the compartment layer from a viscoelastic polymer. On the one hand application of the sample carrier to a base part by simple pressing is made considerably easier as compared to conventional sandwich constructions which operate with mechanical clamping means or laminate connections. On the other hand the material of the compartment layer, in particular when silicon rubber is used, is distinguished by excellent properties such that there is no unspecific adsorption. This is especially significant in the case of miniaturised samples. The sample carrier is inert under interesting reaction conditions when used for medicine, biochemistry and molecular biotechnology. The biologically inert material enables the gripping, cultivation and measuring of biological samples or substrates in the reaction substrates. Finally, the material of the sample carrier can also be cleaned after actual use in a bath or a washing machine using conventional cleaners or solvents, without the form or stability of the sample carrier being adversely affected. The sample carrier can be autoclaved and sterilised without loss of form and without impairment to its adhesive properties. The sample carriers can be reused simply by being removed from the base part.

The reaction substrate according to the present invention comprising base part with superposed sample carrier has particular advantages with respect to the construction of

the reaction substrate, sealing of the sample chambers and the reciprocal orientation of the sample chambers. The sample carrier is applied to the base part by even pressure using a defined, e.g. manually applied pressure. The sample carrier can be used without frames and, with application of adjustment marking, nevertheless enables precise spatial orientation and positioning, for example with respect to a microscope or a sample supply device. The sample chambers, which are formed by penetrating recesses in the compartment layer relative to the base part are sealed without additional sealing or adhesive means. There is no influence of biochemical reactions in the compartments by such means.

The adhesive connection between the sample carrier and the base part and the cover enables planarity also of large-surface reaction substrates or sample carriers having characteristic dimensions of up to 118 mm · 82 mm. Variations of the sample chamber positions in the z direction (perpendicular to the sample carrier plane) can be kept to values less than 250 μm, especially preferably less than 150 μm and particularly preferably less than 100 μm over the entire surface of the base part. This is of particular advantage for microscopic examinations. While a reaction substrate is being measured it is not necessary to continually readjust the z position of the microscope lens. The reaction substrates according to the present invention are thus very well suited to use in testing procedures with high sample throughput (so-called high throughput screening, HTS) in biotechnical and/or chemical research and development, since time-intensive readjusting, e.g. of microscope lenses, is not required in the z direction.

The stability of the reaction substrate is as high as for conventional sample chamber structures, whereby according to the present invention additional adhesive or clamping means can be dispensed with. The stability is substantially increased when the cover is applied.

The reaction substrate has a broad range of application, as a suitable base part can be used as underlay for the sample carrier, in accordance with requirements. The base part can be freely varied with respect to material and thickness. Glass of any thickness, e.g. that of cover glass thickness, preferably serves as a transparent base part, for use in microscopy. The base part can comprise UV-permeable quartz glass. It has outstanding optical properties and is neither chemically modified by the sample carrier nor physically stressed.

The impression tool according to the present invention has the advantage of simple, modular construction. The tool can be adapted by changing the mask or impression shape to any requirement. It is equally suitable for use in the laboratory field or in mass production. The process according to the present invention can be used to manufacture any structures without special expense. This is a particular advantage compared to conventional techniques for structuring glass or semiconductors.

Further details and advantages of the invention are apparent from the description of the attached drawings, in which:

Figure 1 is a schematic perspective view of a reaction substrate with a sample carrier according to the present invention,

Figure 2 is a plan view of a first embodiment of a reaction substrate according to the present invention,

Figures 3, 4 and 5 illustrate an impression tool according to the present invention in the assembled or disassembled state,

Figure 6 is a plan view of a microsample carrier according to the present invention and of a conventional semiconductor structure,

Figure 7 is an enlarged sectional view of a microsample carrier according to Figure 6,

Figure 8 illustrates details of the compartment structures in a sample carrier according to Figures 6 and 7,

Figure 9 is a plan view of other embodiments of a sample carrier according to the present invention with microchannels,

Figure 10 shows a reaction substrate according to the present invention in the form of a layer-shaped fluorescence cuvette,

Figure 11 shows experimental results illustrating the outstanding planarity of reaction substrates according to the present invention, and

Figure 12 shows experimental results illustrating the tightness of compartments of reaction substrates according to the present invention.

The invention is described hereinbelow with respect to a reaction substrate with a microstructured sample carrier for handling biological samples. The invention is not, however, restricted to applications where microscopically small quantities of samples are manipulated in microstructures. Furthermore, the invention is not restricted to the illustrated forms of sample chambers. Depending on application, any other forms with straight or curved walls of the sample chambers can also be realised.

Figure 1 illustrates a reaction substrate with a sample carrier according to the invention in schematic perspective. Various compartment structures and additional devices, which can be provided separately or simultaneously according to use, are shown on the sample carrier. The reaction substrate 100 comprises the base part 10 and the sample carrier 20. The base part 10 is for example a flat glass plate of a thickness corresponding to a thickness in turn corresponding to the thickness of cover glasses for use in microscopy (around 150 µm) and a surface of around 120 mm · 70 mm. The base part 10 can also be formed by any other body with a smooth, even or curved surface. The base part preferably has a substantially smooth plane surface.

The sample carrier 20 comprises a compartment layer 21 (mat) with compartment structures 30. The compartment layer 21 preferably comprises silicon rubber and has a thickness of around 0.5 mm to 4 mm. A flap 22 for removing the sample carrier 20 from the base part 10 and/or adjustment marks 23 for positioning the sample carrier 20 relative to a measuring or sample supply device can be provided on one or more sides of the mat. The adjustment marks 23 are for example punctiform or cruciform recesses in the surface of the sample carrier 20, which are provided if required with an additional marking substance (e.g. fluorescent dye). The adjustment marks have characteristic dimensions, which can be considerably less than the dimensions of the compartment structures 30.

The silicon rubber is e.g. polydimethyl siloxane (PDMS, made by Wacker-Chemie GmbH, ref. M 4600). In general, elastic synthetics (elastomers) can be used which remain elastic at different temperatures. The molecular chains (carbon chains) are cross-linked more loosely in the elastomers, such that the elastomers are rubber elastic. The preferred silicon is a synthetic from the elastomer group and primarily consists of silicon and oxygen. In the

non-cross-linked state silicons are oleaginous, limpid and heat-resistant. In the cross-linked state silicons form a silicon rubber.

The compartment structures 30 individually comprise closed sample reservoirs 31 in the form of throughholes 31a or recesses 31b sunk in the surface of the sample carrier (diameter e.g. ca. 200 µm to 1.5 mm) or straight, curved or branched channels 32 running in the layer plane of the sample carrier. The reference numeral 33 refers to so-called storage pots which are designed similarly to the sample reservoirs 31 for sample uptake and removal, though with larger volumes.

The manipulation and examination instruments 40 comprise for example a fluid line in the form of at least one capillary 41, at least one electrode 42 and/or at least one sensor 43, all arranged in the layer plane of the sample carrier 20, on the walls of the compartment structures 30 or in the compartment structures 30. The capillary 41 can for example be attached to a sample or reagent supply system (not illustrated here). While the sample carrier 20 is being produced (see below) it is embedded therein or is subsequently cut into the sample carrier 20. The electrodes are structured such as is known per se from microsystems technology of micro-electrodes for electro-osmotic pumping procedures, particle handling using negative dielectrophoresis or particle treatment, such as e.g. electroporation of biological cells. The electrodes or their supply lines are preferably embedded in the sample carrier 20 during manufacture, or arranged on its inner surfaces (compartment walls).

Figure 1 also illustrates the cover 50. The cover 50 is not a necessary feature of the reaction substrate according to the present invention. It is provided depending on application and similarly to the base part 10 comprises a

fixed plate (e.g. made of glass) or a flexible cover foil. It can be arranged that the cover 50 has openings 51 corresponding to the positions of the compartment structures 30. The openings 51 aid in supplying sample reservoirs 31 or storage pots 33 or introducing samples into the channels 32. They can be sealed with an additional foil (not illustrated here) as protection against evaporation.

An embodiment of a compartment layer 21 significant for practical applications in biochemistry is illustrated in Figure 2. The compartment layer 21 is a flexible mat made of silicon rubber (e.g. Elastosil M 4600 A + B, manufactured by Wacker, Germany). It has a surface of 118 mm · 82 mm and a thickness of 4 mm. The sample reservoirs 31 (partially illustrated) are arranged in matrix form in straight rows and columns in a 48 · 32 format, each having a mid-point distance of 2.25 mm. This corresponds to the standard format for microtiter plates with 1536 wells. The diameter of each sample reservoir 31 is 1.5 mm. The reference numeral 23 refers to an adjustment marking which is formed in this embodiment likewise by a recess as for the sample reservoirs and can accommodate a reference sample.

The sample carrier 20 or the compartiment layer illustrated in Figure 2 is attached to a base part (not illustrated here) which preferably has the same surface dimension as the compartment layer 21. The base part is preferably a cover glass with a thickness of around 150 µm.

Hereinafter and with reference to Figures 3 to 5 the production of a reaction substrate according to the present invention by casting of the compartment layer in an impression tool is explained. The figures show the impression tool in a perspective phantom view or disassembled in perspective or side view. The impression

tool 200 basically comprises a closed container with an inner cavity corresponding to the outer shape of the desired compartment layer, or with inner surfaces with protrusions corresponding to the desired compartment structures. For the widest possible use the container is designed modular from a base plate 60, an intermediate plate 70 and a counter plate 80, all able to be connected to one another in a liquid-tight manner. The base, intermediate and counter plates are preferably connected to one another detachably.

On its side facing the interior of the impression tool 200 the base plate 60 bears projections for structural formation in the compartment layer. Apart form the projections the surface of this inner side is designed uniform and smooth. In the illustrated example the projections comprise pins 61 arranged in a matrix (partially illustrated) with a diameter corresponding to the desired diameter of the sample reservoirs 31 (see Figure 2). The pins 61 are stuck into corresponding recesses on the inside of the base plate 60. The base plate and the pins preferably comprise metal (e.g. stainless steel or aluminium). However, other materials such as e.g. silicon or glass can also be used for the projections for structural forming. These materials can be processed using special shaping techniques known per se (e.g. LIGA process or etching) with high precision into the sub-micrometer range, whereby the resulting projections can reach heights of up to 1 mm. The base plate 60 can have a separate mask insert for mounting the projections (metal pins or other structures). Figure 4 also illustrates the metal pin 61a, provided for forming the adjustment marking 23 (see Figure 2).

The intermediate plate 70 is a spacer which determines the thickness of the compartment layer (silicon mat) and whose internal dimension establishes the external dimension of

the compartment layer. The intermediate plate 70 is fitted with a filling opening 71 which cooperates with the filler neck 90 (see below), and outlet openings 72. The outlet openings 72 aid in releasing displaced air or excess layer material from the impression tool 200. The intermediate plate 70 is not a coercive feature of an impression tool according to the present invention. The function of the spacer can alternatively also be fulfilled by corresponding structures (revolving steps) on the base plate and/or the counter plate.

The counter plate 80 constitutes the seal of the impression tool 200 relative to the base plate 60. It is likewise a metal plate. Arranged facing the inside of the impression tool 200 in the counter plate 80 is a frame 81 with a synthetic insert 82. The synthetic insert 82 is a layer of elastically deformable synthetic material having a thickness of ca. 10 mm. It preferably comprises PTFE. The synthetic insert 82 has recesses 83, complementary to the projections on the base plate 60. In the illustrated example 1536 boreholes (partially illustrated) for taking up the metal pins 61 when the impression tool 200 is in the assembled state are provided in the synthetic insert 82. Building in the complementary recesses is not absolutely necessary. If the projections on the base plate 60 are sufficiently stable or the synthetic insert 82 can be sufficiently easily deformed, so that when the impression tool 200 is in the assembled state the projections are not damaged, then separate recesses in the synthetic insert 82 can be dispensed with.

The reference numeral 20 refers to the finished sample carrier (according to Figure 2), which is produced with an impression tool 200 according to Figures 3 to 5.

According to the present invention it can be arranged that the recesses 83 in the synthetic insert 82 are bored

completely through it and also continue in corresponding recesses 84 in the counter plate 80. These openings serve as an exit for displaced air or excess layer material.

The filler neck 90 is fastened externally on the assembled impression tool 200 at the filling opening 71. It serves to introduce the dissolved liquid polymer mass into the assembled tool form.

The impression tool 200 is assembled using mounting bolts 62, 63, 64, 65 which pass through corresponding bores at the corners of the base, intermediate and counter plates. A screw connection (not illustrated separately) is provided for fastening the components together. Alternatively, external clamping devices or a separate frame for holding the plates together can be provided.

The impression tool 200 can be modified as follows. In addition, a metallic frame, which has the desired external dimension of the compartment layer and also remains connected thereto during later use, can be accommodated inside the intermediate plate 70. At their ends the pins 61 can be rounded to facilitate insertion into the corresponding recesses in the base or counter plate. For integrating the manipulation and examination instruments named in reference to Figure 1 in the sample carrier 20 it can be arranged to accordingly provide the intermediate plate 70 with mountings for these additional devices. These mountings may comprise leadthroughs in the frame formed by the intermediate plate 70 from the inside of the impression tool 200 outwards, which are each equipped with fittings (e.g. clamps) for the respective additional devices. Finally, it is not absolutely necessary that all structures of the desired compartment layer are actually designed as projections on the base plate 60. The finished sample carrier can still be provided with additional structures (e.g. bores for the storage pots 33).

For producing the sample carrier the impression tool 200 is first put together. The pins 61 are stuck into the base plate 60. The base, intermediate and counter plates are assembled such that the pins 61 project into the recesses 83 in the synthetic insert 82. In this way the result is a container essentially closed to all sides, between whose side plates (base and counter plates) the pins 61 extend. The guide pins 62 to 65 are e.g. tightened using wing nuts. The assembled tool is placed upright with vertically directed plates. The filling opening 71 points upwards.

The impression tool 200 is then filled through the filling opening 71 with a solution of the required polymer composition. This is preferably injected directly into the filling opening 71 or using the filler neck 90. Filling takes place as slow feeding, avoiding splashes or swirls so that the inside of the impression tool 200 is filled as uniformly as possible. Filling continues until the dissolved polymer composition overflows out the outlet openings 72. These are then closed off with an adhesive strip, for instance. After this sealing a little more material is filled in.

The polymer composition is then dried at room temperature. This can last approximately 8 to 12 hours, for instance. Removal of the solvent or cross-linking polymer composition can be accelerated by tempering. Finally, the compounds of the plates are loosened by the guide pins 62 to 65, the plates are separated from one another and the elastic compartment layer drawn from the mask or impression shape. A particular advantage of the use of silicon rubber here is that this extraction can take place without problems and without damage to the sample carrier.

Cross-linking occurs by means of the polymer Elastosil M 4600 preferably at room temperature, though this can be

carried out at higher temperatures in a drying cabinet or an oven. This cross-linking is essentially chemical cross-linking, wherein a polymerisation reaction occurs in the presence of a catalyst, if required. With other polymers cross-linking takes place at the respective specified cross-linking temperature.

Final processing for subsequent placing of compartment structures (e.g. storage pots) or for adaptation or alignment of additional manipulation and examination instruments can follow. Subsequent chemical processing of the surface of the sample carrier is also feasible. The finished sample carrier is then set on a base part and attached thereto by simple manual pressure.

Another embodiment of the invention as an example of a micro-sample carrier for the smallest quantities of liquid is illustrated in Figures 6 to 8. Figure 6 first shows a size comparison between a sample carrier 20 according to the present invention (left part of diagram) and a conventional sample carrier 20' which is made of silicon. On a base surface of around 10 mm · 15 mm the sample carrier 20 bears a matrix arrangement of a total of 600 funnel-shaped compartments (see below). Each compartment has a characteristic cross-section dimension of around 0.5 mm. By way of comparison the conventional silicon sample carrier 20' has a considerably larger grid which was produced separately using expensive processing techniques.

Figure 7 illustrates an enlarged section of the sample carrier 20. This image was recorded with an inverse microscope with a CCD camera. The sample carrier 20 bears the compartments 34 arranged in straight rows and columns. They have a cross-sectional form tapering from the surface of the sample carrier 20 into the compartment layer in the manner of a reverse truncated pyramid. At the base the compartments have a characteristic side length which is

approximately 1/3 of the upper edge length. The lightly illustrated base is formed by the common base part 10 (see Figure 1). The compartments completely pass through the compartment layer 21 of the sample carrier 20.

A sample carrier according to Figures 6 to 8 is produced with a correspondingly adapted impression tool in a similar manner to the process described with reference to Figures 3 to 5. In the impression tool the projections on the base plate are formed not by inserted pins, but in the form of a pyramid by mechanical milling. After it is manufactured compartment layer 21 is attached to a glass base part by adhesion. The compartments are filled and then if necessary sealed off with another glass component as a cover or with a foil. The microscopic measuring of the samples in the compartments is performed from the side of the base part 10 through the lower smaller openings of the compartment layer 21. The edge length of the lower openings is respectively around 150 µm.

Figure 8 illustrates details of the frames formed between the compartments. As is also evident from Figure 7 the compartment layer is formed such that the walls between the compartments 34 form continuous frames 35 in the row direction and interrupted frames 36 in the column direction. An overflow 37 is formed between the ends of the interrupted frames 36 and each of the contiguous continuous frame 35. The overflow 37 enables production of a liquid connection between adjacent compartments without infringing over the upper surface of the sample carrier 20. The arrangement of the overflows can be modified depending on the application.

Figure 9 illustrates different forms of channel structures in a sample carrier according to the present invention on an enlarged scale. The channels 32 in the layer plane are generally open sample chambers or compartment structures,

whose expansion in one direction is considerably greater than in a direction perpendicular thereto. Channels are formed in the sample carrier, in that a mask form with frame-shaped projections on the base plate of the impression tool is used for their manufacture. The channels can run straight or curved individually or be branched or interconnected. Depending on the shape of the sample carrier even self-contained channels can be formed, in case the channel floor itself is part of the sample carrier, while the corresponding compartment structures therefore do not go right through the compartment layer.

Figure 9A illustrates a channel structure with several channels 32a to 32c which are connected to a mixing cross 32d. On each of the channel ends are storage pots 33a to 33d. Reference numeral 32e refers to a throat. The throat 32e can be formed in terms of fluid mechanics by barriers (bulged channel wall) or also electrically by electrical field barriers, for example for retarding the liquid flow before it reaches this region and to take measurements there of suspended particles in the liquid flow.

Figure 9B shows a variation. Two partial channels 32a, 32b are connected into a common channel 32c. This structure serves to mix together two liquid flows into a single liquid flow. The angle α between the partial channels 32a, 32b is adjusted depending on use to obtain a uniform flow at the mixing point 32d. Another variation of structures for mixing liquid flows is illustrated in Figure 9C as double cross arrangement with several partial channels which terminate in two mixing points 32d.

The meandering form 32f according to Figure 9D aids in creating a particularly long measuring path. Extending between the storage pots 33a to 33c on one side and the storage pot 33d is a long, helical channel in a surface

area which forms a target for illuminating fluorescent measurements.

The reaction substrates or sample carriers according to the present invention have particular advantages with respect to the formation of the channel structures. For making the mask for the impression tool any channel courses can be prepared using conventional precision mechanical tools (e.g. CNC machines) from a common material, preferably aluminium or other metallic substances. They can be formed in particular with respect to length, relative orientation (angle), bends and windings, compound structures and part channels in a predetermined manner depending on use. Channels of this type can be manufactured precisely and reproducibly right up to channel widths of around 6 μm using conventional precision mechanical tools. Projections or edges can be machined into the channels, enabling improved mixing of several liquid streams when several channels are joined together. The channels can be fitted with electrodes for measuring the properties of the liquid streams or with sensors or tempering elements and also with retaining or valve elements and piezopumps for manipulating on the basis of electro-osmosis.

Another embodiment of the invention with a macroscopic compartment structure is illustrated in Figure 10 in plan and sectional views.

A sample carrier 20 according to the present invention can also be equipped with a single chamber compartment 38. The compartment layer 21 is merely a ring made of the polymer composition used, e.g. silicon rubber. This ring adheres between a base part 10 and a cover 50, thus forming a closed, layer-shaped cuvette e.g. for fluorescence spectroscopy. Due to the liquid-permeable adhesion of the sample carrier 20 to the glass materials of the base part 10 or the cover 50 this cuvette can be supplied permanently

with solvents or sample solutions and subjected to fluorescence measurements in the manner of a fixed layer sample.

Figures 11 and 12 depict particular advantages of reaction substrates according to the present invention with respect to planarity of the sample arrangement, of significance for microscopic examinations, with well-to-well tightness of the compartment structures. For demonstrating the planarity the variation of the z position over the entire area of the base surface of the reaction substrate was measured using a confocal microscope (reflection of the laser beam on the glass surface of the base, taken with a CCD camera) in a conventional, commercially available reaction substrate or sample carrier (left partial image in Figure 11) and for a reaction substrate according to the present invention (right partial image in Figure 11).

With conventional, commercially available sample carriers or reaction substrates there is a variation no longer tolerable for confocal-fluorimetric applications of the plate base in the z direction by up to 300 µm from the edge of the plate to its centre. A definite course in one direction ("sagging" of the reaction substrate in its middle) is to be recognised. The reaction substrate, which is the subject of this application, shows a variation of the plate base in the z direction of far less than 100 µm. This deviation is clearly below the tolerance of around 150 µm for confocal-fluorimetric applications. Furthermore, only a statistical fluctuation of the z deviation by the average value is to be recognised up or down, and there is no tendency in the deviation (e.g. no "sagging" of the reaction substrate in its middle).

Figure 12 shows the 1536 wells of a reaction substrate according to the present invention with the results of measurements taken in each of the wells. For this test a

reaction substrate in the form of a "chess board pattern" was filled alternatingly with suspensions of so-called active and so-called inactive bacteria (each 330 nl per well). After an incubation period of 20 hours all wells were treated evenly with 1 µl assay each; following a further incubation period of 30 minutes all wells of the reaction substrate were measured using CFCA measurements (1 second measuring time per well).

In wells which are treated with active bacteria the two-tone assay molecules are split so that the CFCA signal diminishes (black fields in the plot). In samples which are treated with inactive bacteria the two-tone assay molecules are not split so that the CFCA signal remains strong (white fields in the plot). In only a total of six out of 1536 wells an "error" occurs which could originate from leakage between individual wells. There could also be errors which have already arisen form pipetting of the bacteria suspensions. The upper limit for errors occurring from leakages from well to well is 0.4% at the most.

This excellent result is beneficially also time-stable. The reaction substrate is also stable after at least 40 hours (time for preparing the samples via incubation until measuring is completed) in the sense that the wells are closed off against one another and measurements can be taken in the plate (without adhesion of the base glass which can again be removed when measurements are completed).

The result illustrated in Figure 12 also points out that the growth of bacteria is not prevented by the compartment layer (biocompatibility).

The sample carriers or reaction substrates according to the present invention can generally be applied in all fields of biochemistry, biology or molecular biotechnology, where one

or more samples are to be held, handled or altered in a defined form. Preferred uses are the processing of suspensions with certain particle mixtures. For example, cell sorters, molecule sorters or other cell manipulators can be out together using reaction substrates according to the present invention. All applications of fluid microsystems technology can be implemented.

The reaction substrates according to the present invention can be used with particular advantage in synthesis processes which are based on combinatory chemistry. In particular, the reaction substrates according to the present invention can be used for identifying and validating targets, that is, specific biological molecules, such as enzymes, receptors or ion channels. Moreover they can be used highly effectively for identifying biologically active substances and/or pharmaceutical materials. Through the possibility of using the reaction substrates according to the present invention in testing with high sample throughput (so-called high throughput screening, HTS) clearly more substances can be examined within a short time with respect to their biological activity and/or pharmaceutical efficacy. This is of particular significance for examining the substance banks obtained by means of combinatory chemistry with respect to their biological activity and/or pharmaceutical efficacy. With the reaction substrates according to the present invention it is possible to achieve a considerable sample throughput and to examine between several thousand up to 10,000 substances per day.

The reaction substrates according to the present invention are also highly suitable for performing assay procedures. In these assay procedures targets and chemical compounds are combined for testing chemical and/or biological interactions. Accordingly, a model system can easily be set up allowing substances to be identified which influence the

target in the intended manner. The reaction substrates according to the present invention can be used both for biochemical and cellular assay procedures. Included here also are assay procedures based on the use of vesicular particles or solid particles (so-called beads).

The reaction substrates according to the present invention are also highly suitable for performing assay procedures, based on using simplified model systems, which imitate the physiology in humans or in animals. This means that the assay systems can be used inter alia for obtaining information on the solubility of biologically active and/or pharmaceutically effective substances in blood plasma, their penetration properties, their liver toxicity, their bioavailability, their stability in blood or their decomposition profile after passing through the liver.

The chemical and biotechnical examinations can be used for example i) for identifying and characterising synthetic or biological objects, ii) for identifying and characterising chemical compounds, iii) for identifying and/or validating targets, iv) for searching for biologically active substances and/or pharmaceutical materials, v) for identifying conductive structures, vi) for genome analysis, vii) for proteome analysis, viii) for cleaning and concentrating substrates, or ix) for evolutive optimising of biologically relevant macromolecules.

15008/US Hz/hr

CLAIMS

1. A reaction substrate (100) having a compartment structure (30), by means of which sample reservoirs (31) are formed, arranged like a matrix in straight rows and columns, characterised by a base part (10) comprising a glass, plastic, metallic or semiconductor substrate and having a substantially plane smooth surface, and a flexible compartment layer (21) made of a polymer material, in which the sample reservoirs (31) are formed, whereby the polymer material is a viscoelastic polymer composition which has an inherent adhesion with respect to the surface of the glass, plastic, metallic or semiconductor substrate and can be separated from the base part (10) substantially free of damage without loss of form, adhesion and flexibility, whereby the compartment layer (21) is completely penetrated by the sample reservoirs (31), such that the surface of the base part lies free on the floors of the sample reservoirs (31).

2. The reaction substrate as claimed in Claim 1, wherein the base part comprises a transparent material.

3. The reaction substrate as claimed in Claim 2, wherein the base part (10) is a substantially plane smooth glass plate.

4. The reaction substrate as claimed in Claim 3, wherein the glass plate has the thickness of a cover glass used in microscopy.

5. The reaction substrate as claimed in Claim 4, in which the thickness of the cover glass is around 150 µm.

6. The reaction substrate as claimed in any one of the foregoing claims, wherein the polymer composition

comprises natural or synthetic rubbers free of adhesive and solvent.

7. The reaction substrate as claimed in Claim 6, wherein the polymer composition comprises silicon rubber.

8. The reaction substrate as claimed in Claim 6, wherein the polymer composition sticks to the base part (10) free of adhesive.

9. The reaction substrate as claimed in any one of the foregoing claims, wherein the compartment layer (21) can be separated substantially free of damage from the base part (10), without loss of form, adhesion and flexibility.

10. The reaction substrate as claimed in any one of the foregoing claims, wherein the compartment layer (21) bears a cover (50) on the side opposite to the base part (10).

11. The reaction substrate as claimed in Claim 10, wherein the cover (50) has penetration openings (51) for supplying the compartment structures (30) with liquid samples or for removing such samples.

12. The reaction substrate as claimed in any one of the foregoing claims, wherein the compartment structures (30) apart from the sample reservoirs (31) comprise channels (32) and/or storage pots (33).

13. The reaction substrate as claimed in any one of the foregoing claims, wherein manipulation and examination instruments (40) comprising fluid lines (41), electrodes (42) and/or sensors (43) are provided in the compartment layer (21).

14. The reaction substrate as claimed in any one of the foregoing claims and forming a micro or nanotiter plate.

15. The reaction substrate as claimed in any one of the foregoing claims, wherein variations of the sample reservoir positions in a direction vertical to the plane of the reaction substrate over the entire surface of the base part are less than 250 µm, preferably less than 150 µm and particularly preferably less than 100 µm.

16. The use of a reaction substrate as claimed in any one of Claims 1 to 15:
   - for identifying and characterising synthetic or biological objects,
   - for identifying and characterising chemical compounds,
   - for identifying and/or validating targets,
   - for searching for biologically active substances and/or pharmaceutical substances,
   - for identifying conductive structures,
   - for genome analysis,
   - proteome analysis,
   - for cleaning and concentrating substrates, or
   - for evolutive optimising of biologically relevant macromolecules.

ABSTRACT

A reaction substrate comprises a base part and a flexible compartment layer made of a polymer material with preset compartment structures for forming sample compartments, whereby the polymer material is a viscoelastic polymer composition (e.g. silicon rubber) which has inherent adhesion with respect to glass, plastic, metallic or semiconductor substrates. A tool for manufacturing the reaction substrate is also described.

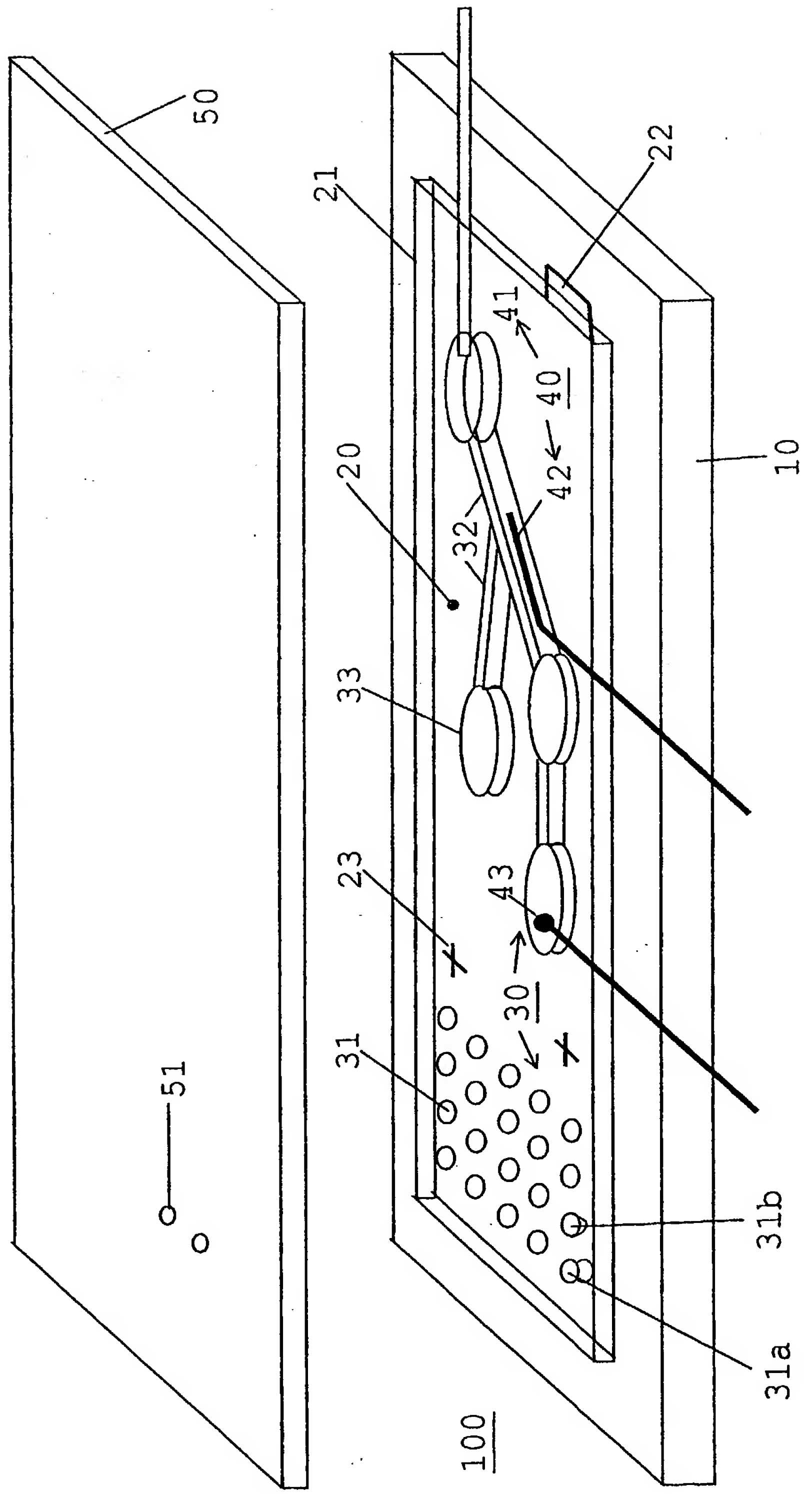


Fig. 1

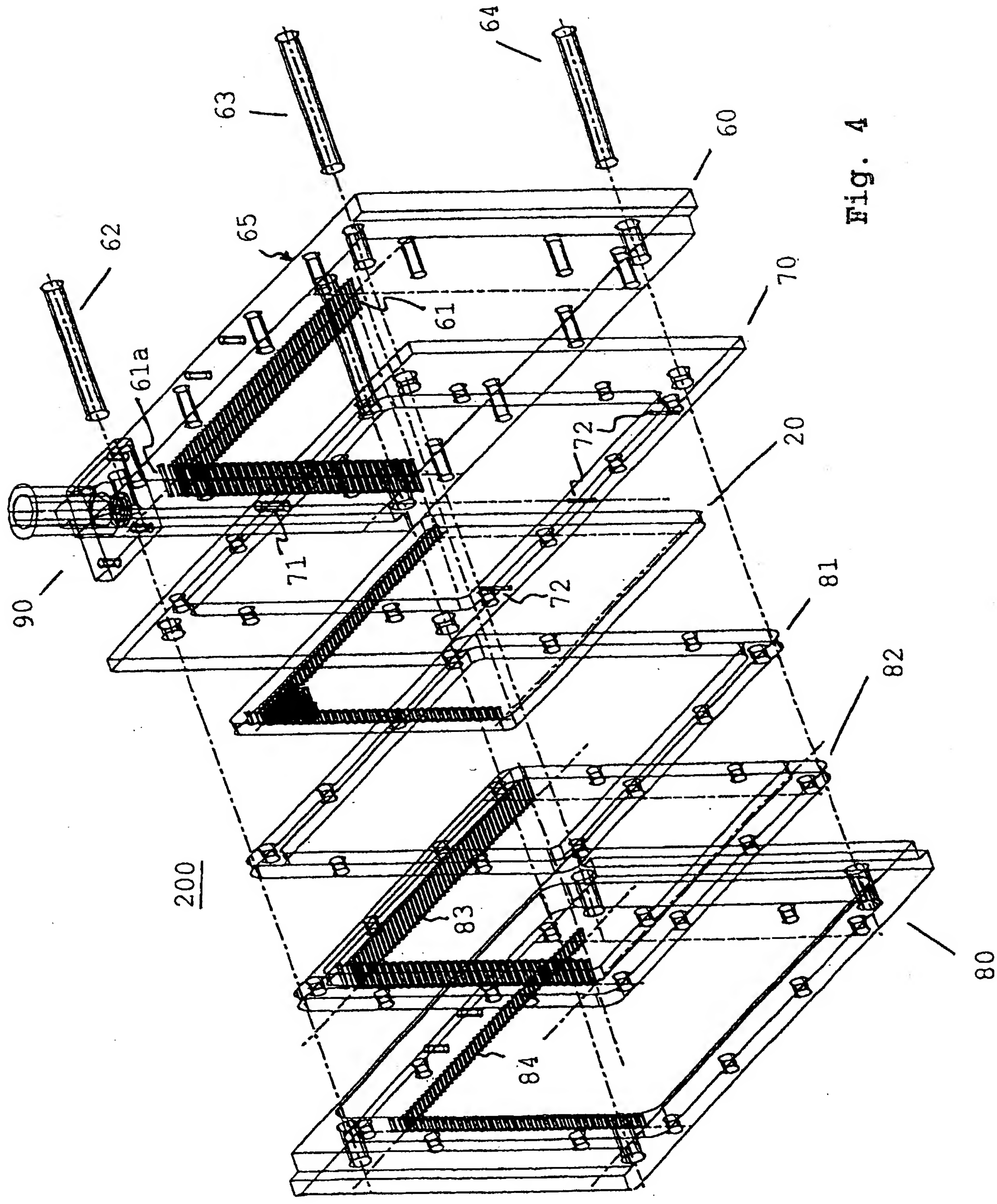


Fig. 4

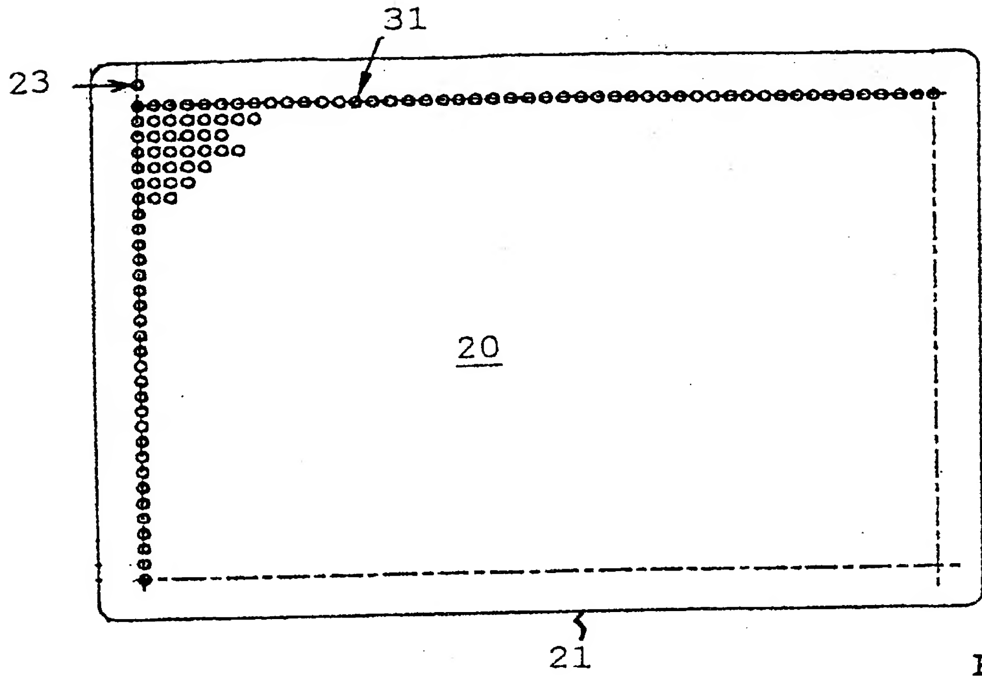


Fig. 2

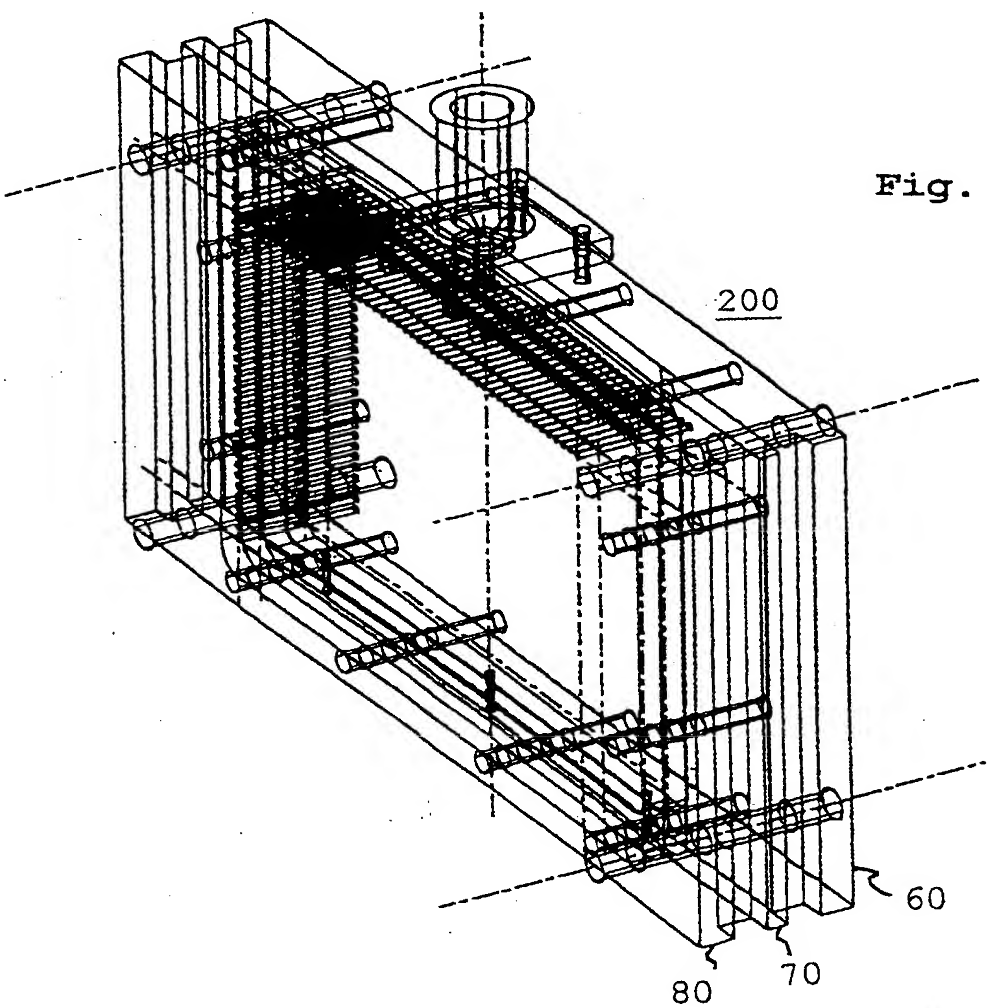


Fig. 3

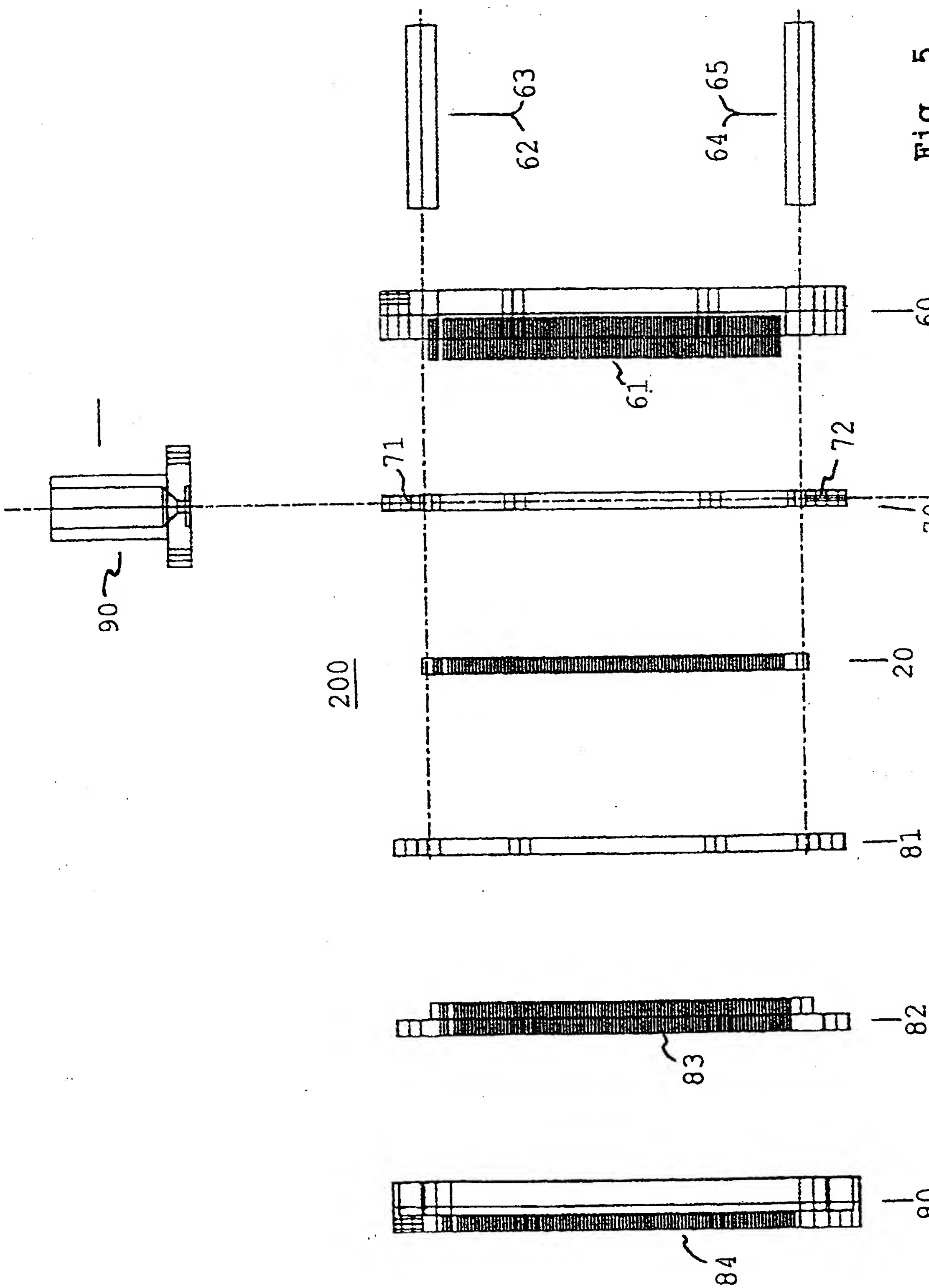


Fig. 5

20'

20

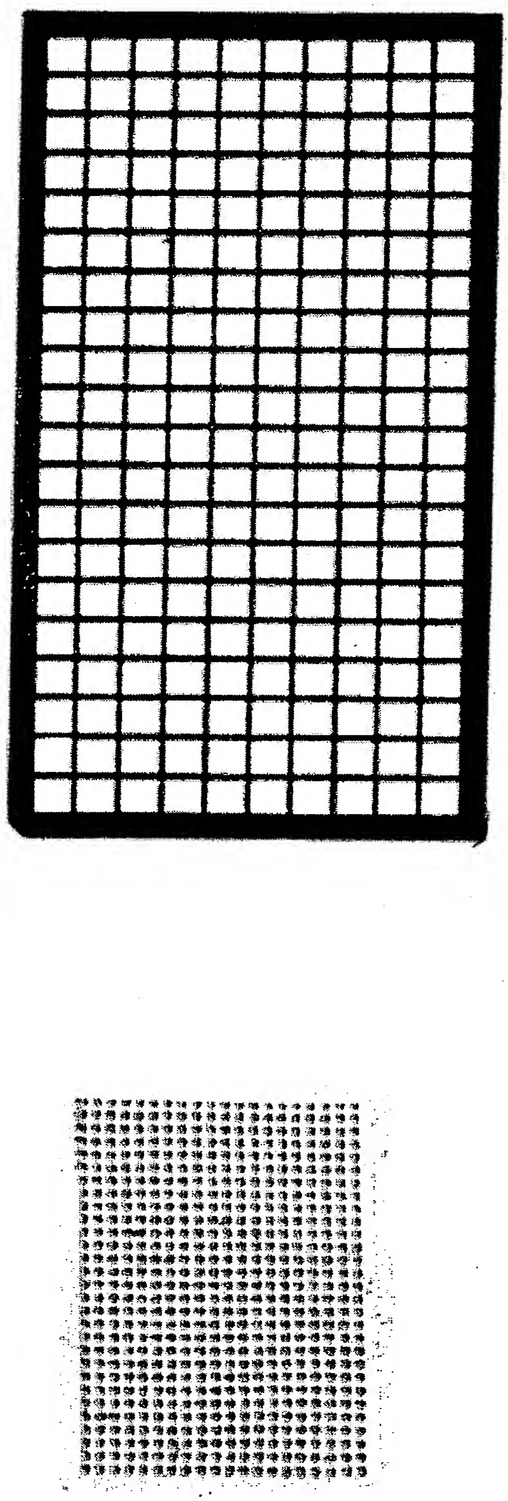


Fig. 6

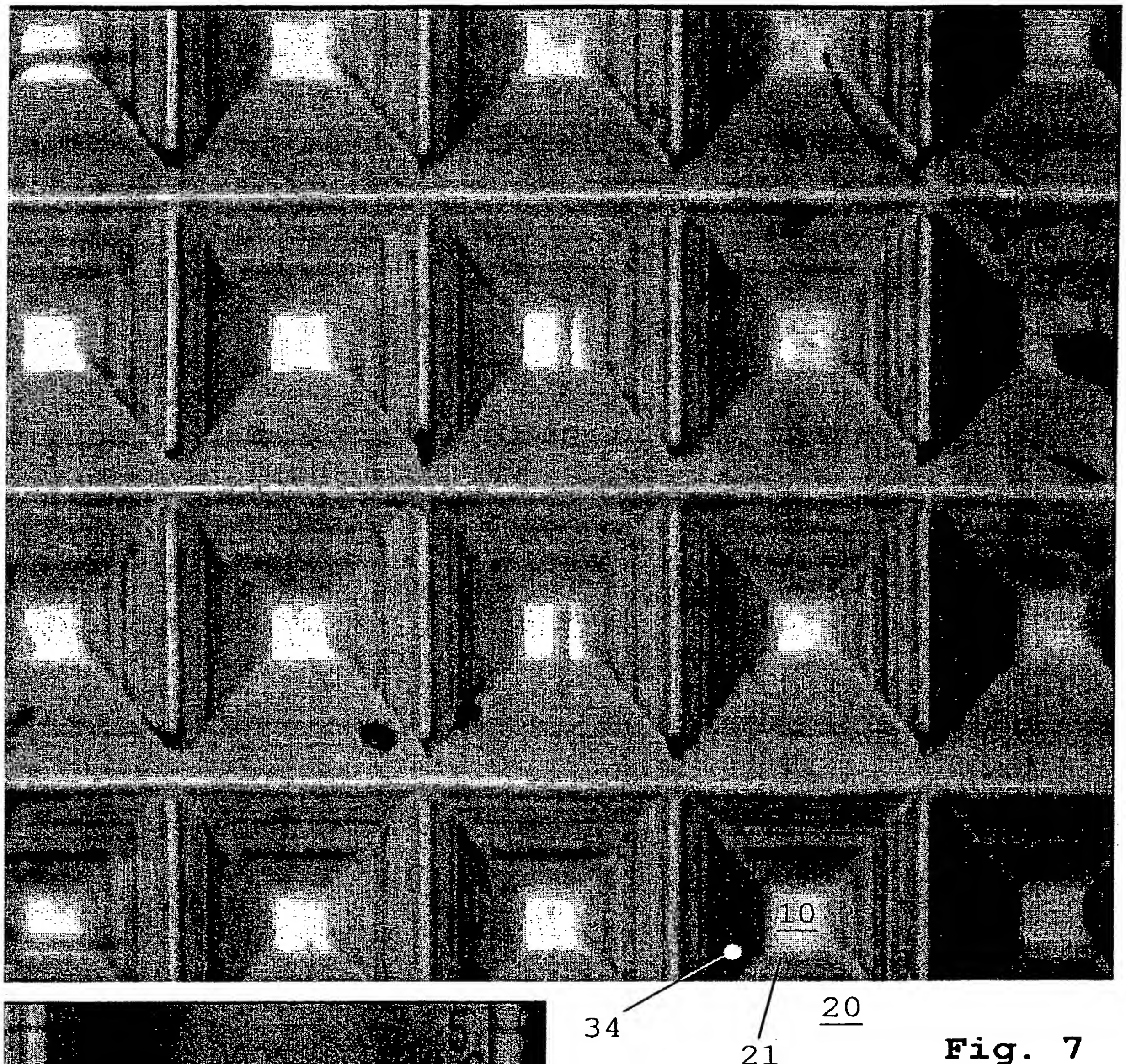


Fig. 7

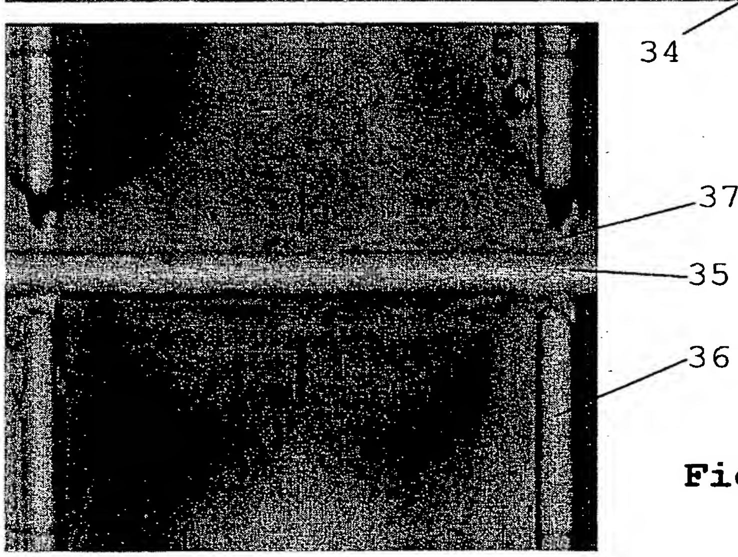


Fig.8

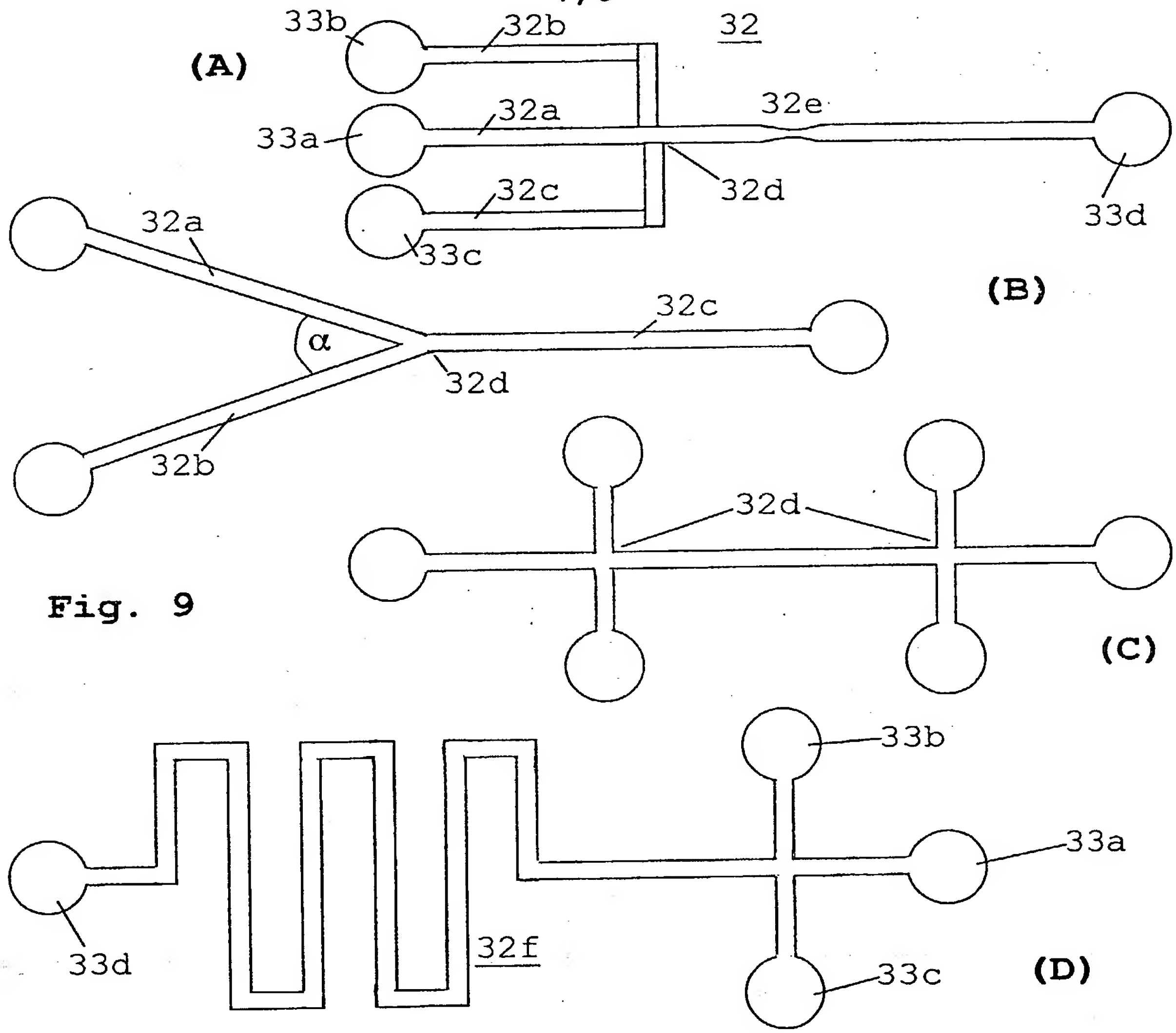

32
(A)
33b
32b
33a
32a
32e
32d
33c
32c
33d
(B)
32a
α
32c
32d
32b
32d
(C)
33b
33a
33d
32f
33c
(D)

Fig. 9

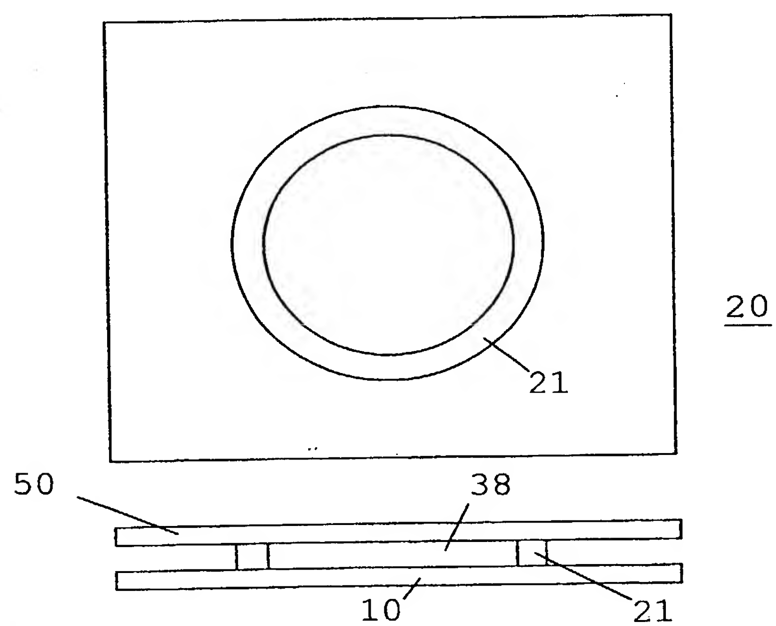
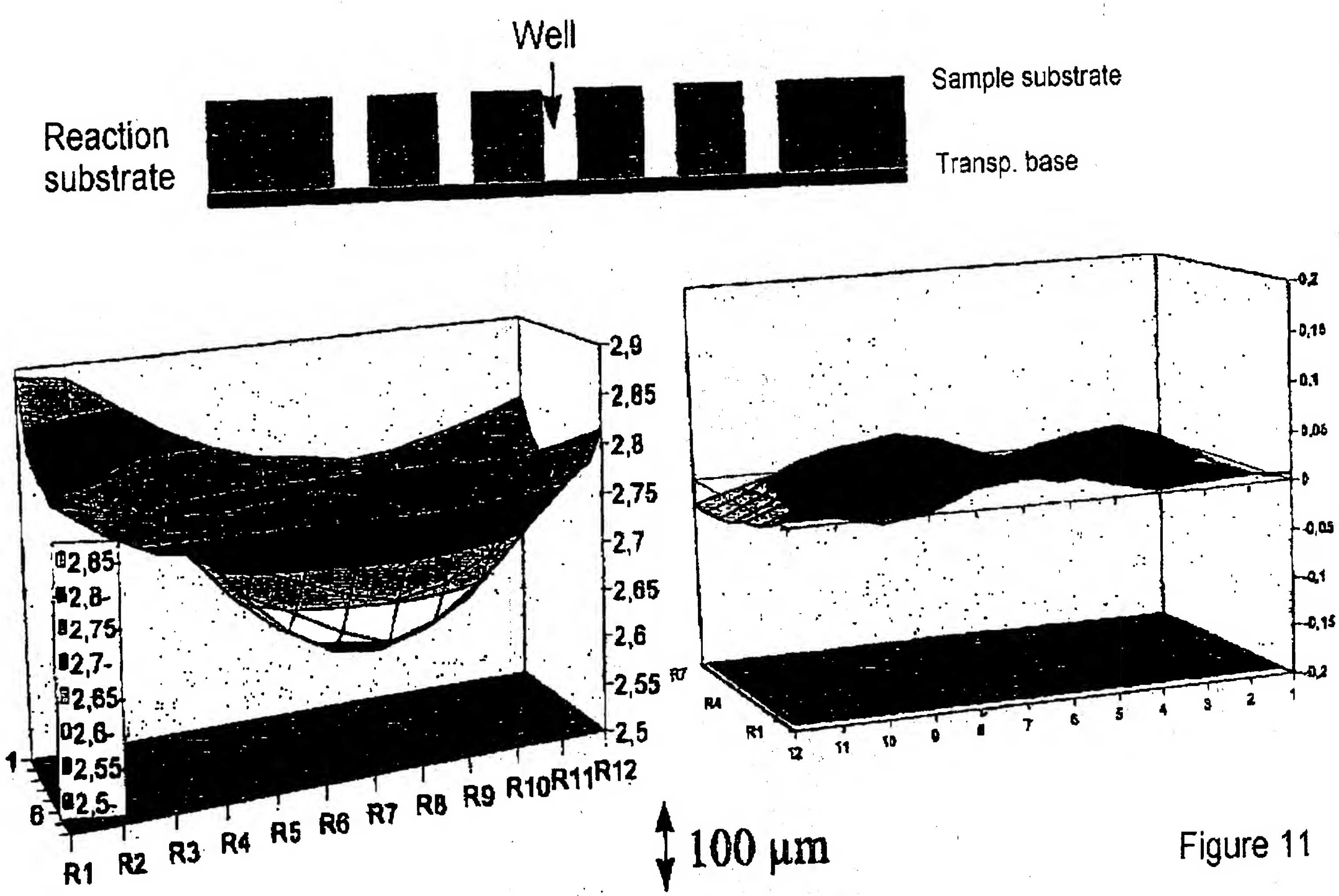

20
21
50
38
10
21

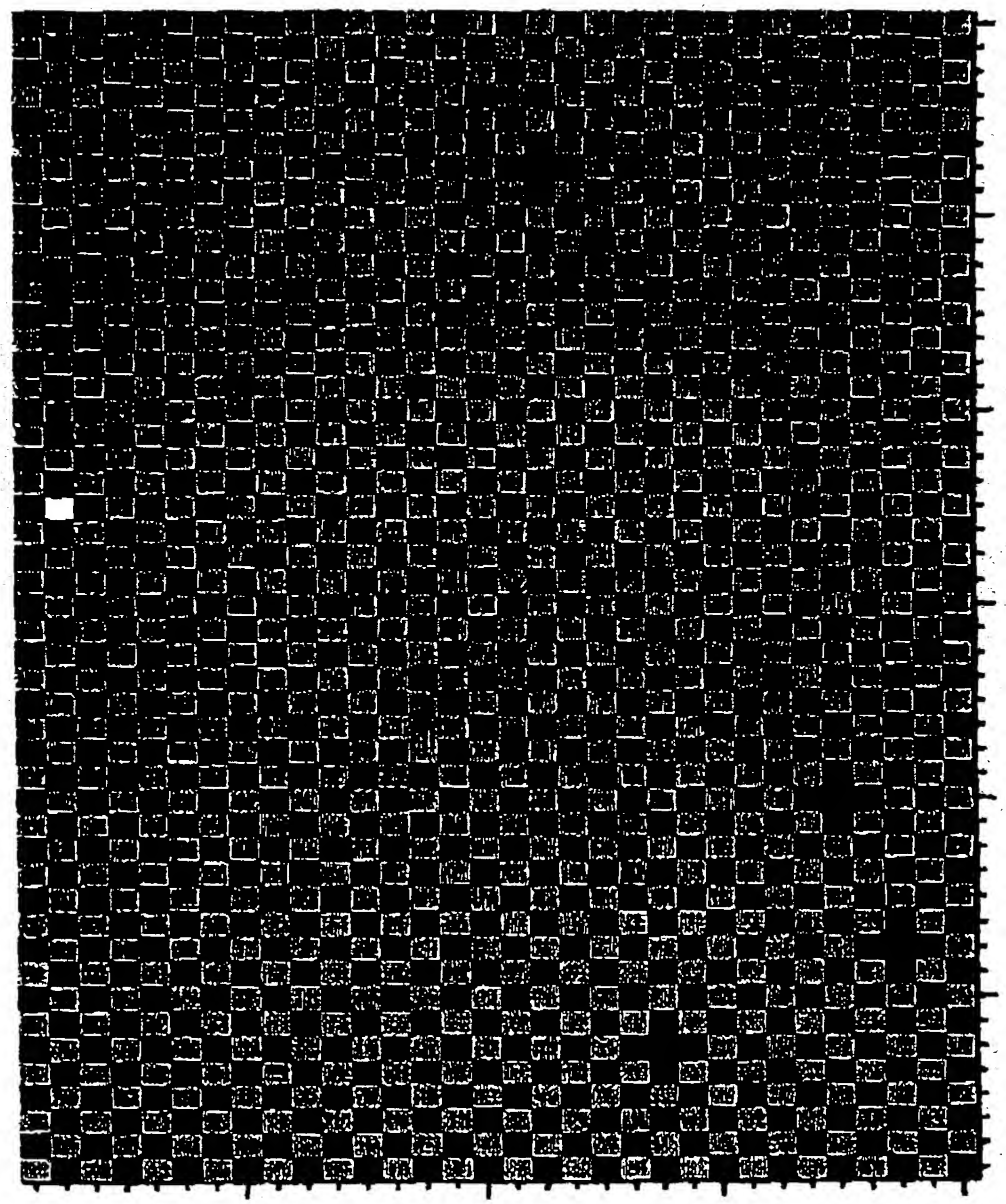
Fig. 10



Conventional sample substrate

Reaction substrate according to the present invention

Figure 11



black: active bacteria detected

white: no active bacteria detected

Figure 12

10089841.052302

Attorney Docket No. B1180/20005

Substitute for Forms PTO/SB/01A (10-01) and PTO/SB/81 (02-01)
CRBCP 02-27-2002

DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention: STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME

As the below named inventor(s), I/we declare that:

This declaration is directed to:

___ The attached application, or
X Application No. 10/089,841, filed on April 4, 2002,
as amended on ______________ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/ we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

I hereby appoint the practitioners at Customer No. 03000 as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith. Please change the correspondence address for the above-identified application to Customer No. 03000.

03000
PATENT TRADEMARK OFFICE

FULL NAME OF INVENTOR(S)

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Inventor three: WOLFGANG SIMM

Signature: ______________ Citizen of: Germany

Inventor four: ULRICH KETTLING

Signature: Ul Kettl Citizen of: Germany

X Additional inventors are being named on 1 additional form(s) attached hereto.

10089841 .093002

Attorney Docket No. B1180/20005

Substitute for Forms PTO/SB/01A (10-01) and PTO/SB/81 (02-01)
CRBCP 02-27-2002

DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention: STRUCTURED REACTION SUBSTRATE AND METHOD FOR PRODUCING THE SAME

As the below named inventor(s), I/we declare that:

This declaration is directed to:

___ The attached application, or
_X_ Application No. 10/089,841, filed on April 4, 2002,
as amended on ____________ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/ we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

I hereby appoint the practitioners at Customer No. 03000 as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith. Please change the correspondence address for the above-identified application to Customer No. 03000.

03000
PATENT TRADEMARK OFFICE

FULL NAME OF INVENTOR(S)

Inventor one: SUSANNE BRAKMANN
Signature: ____________ Citizen of: Germany

Inventor two: HELMUT PEUKER
Signature: ____________ Citizen of: Germany

Inventor three: WOLFGANG SIMM
Signature: Wolfgang Simm Citizen of: Germany

Inventor four: ULRICH KETTLING
Signature: ____________ Citizen of: Germany

_X_ Additional inventors are being named on _1_ additional form(s) attached hereto.

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Attorney Docket No. B1180/20005

Substitute for Forms PTO/SB/01A (10-01) and PTO/SB/81 (02-01)
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PATENT TRADEMARK OFFICE

FULL NAME OF INVENTOR(S)

Inventor one: SUSANNE BRAKMANN
Signature: Susanne Brakmann — Citizen of: Germany

Inventor two: HELMUT PEUKER
Signature: ______________ — Citizen of: Germany

Inventor three: WOLFGANG SIMM
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Inventor four: ULRICH KETTLING
Signature: ______________ — Citizen of: Germany

_X_ Additional inventors are being named on _1_ additional form(s) attached hereto.

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03000
PATENT TRADEMARK OFFICE

FULL NAME OF INVENTOR(S)

Inventor one: SUSANNE BRAKMANN

Signature: ______________ Citizen of: Germany

Inventor two: HELMUT PEUKER

Signature: Helmut Peuker Citizen of: Germany

Inventor three: WOLFGANG SIMM

Signature: ______________ Citizen of: Germany

Inventor four: ULRICH KETTLING

Signature: ______________ Citizen of: Germany

_X_ Additional inventors are being named on _1_ additional form(s) attached hereto.

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Inventor six:	THORSTEN WINKLER		
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Inventor seven:	MANFRED EIGEN		
Signature:		Citizen of:	Germany
Inventor eight:	JENS STEPHAN		
Signature:		Citizen of:	Germany
Inventor nine:	KLAUS DÖRRE		
Signature:		Citizen of:	Germany

FULL NAME OF INVENTOR(S) - CONTINUED

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Inventor seven: MANFRED EIGEN

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Inventor eight: JENS STEPHAN

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FULL NAME OF INVENTOR(S) - CONTINUED

Inventor five: ANDRE KOLTERMANN

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Inventor seven: MANFRED EIGEN

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FULL NAME OF INVENTOR(S) - CONTINUED			
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Signature:		Citizen of:	Germany
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Inventor eight:	JENS STEPHAN		
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Inventor nine:	KLAUS DÖRRE		
Signature:	Klaus Dörre	Citizen of:	Germany

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☑ ***Scanned copy is best available.*** Some drawings are dark.